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**USE OF A DIALKYL KETONE PEROXIDE AS A BIOCIDES: STERILIZING,
ANTISEPTIC, DISINFECTING AND ANTI-PARASITIC AGENT**

FIELD OF THE INVENTION

The invention is related to novel biocidal agents, specifically with the use of dialkyl ketone peroxide as a non-toxic, non-ecotoxic, sterilizing, antiseptic, disinfecting and anti-parasitic agent, in all areas of use such as medicine, veterinary sciences, industry, the home, etc. In particular, the invention contemplates the use of dialkyl ketone peroxide as a sterilizing, antiseptic, disinfecting and anti-parasitic agent, the use of sterilising, antiseptic, disinfectant or parasitocidal compositions comprising said dialkyl ketone peroxide, and methods of sterilisation and disinfection that involves the application of such compositions.

BACKGROUND OF THE INVENTION

For several years the techniques of sterilisation by physical or chemical methods have become well known to experts in the field. Of the former, the application of heat or radiation and the use of filters should be highlighted. Of the latter, the use of chemical agents, either antiseptics or disinfectants, and/or sterilising agents, stand out.

The application of humid heat, for example using an autoclave, is a widely used method to destroy bacteria and spores in a short period of time, which does not leave any toxic residues, does not deteriorate the exposed material and is economical. However, it does present some inconveniences in as much as it does not permit solutions forming emulsions with water to be sterilised, it is corrosive to certain metallic instruments and damages instruments that are sensitive to heat, especially if they contain certain polymeric materials.

The application of dry heat, using a sterilising oven or through incineration, for example, is also used for disinfection, as it is not as corrosive to metallic instruments and it permits the sterilisation of substances in powder and non-

aqueous material, as well as non-volatile viscous substances. However, this method requires a longer sterilisation time with respect to the application humid heat and furthermore, it still damages certain polymeric materials.

5 The application of ionising radiation is an economical method used to sterilise thermolabile materials. However, it cannot be used for culture medium or protein solutions because it produces alterations of their components. The application of ultraviolet radiation (weakly penetrating) is used to sterilise surfaces.

10 In respect to sterilisation by filtration, using filter membranes with a determined pore size is a method applied to oily emulsions or thermolabile solutions. However, the filters that are generally used in laboratories do not retain viruses or mycoplasmas.

15 Among the chemical compounds employed, we find sterilising agents, disinfectants and antiseptics. The effectiveness of these agents depends on their concentration and pH, as well as on the time over which they are applied.

20 Among the antiseptic agents, the alcohols, iodine, ionic and amphoteric agents, organo-mercurial compounds and some colorants should be mentioned.

25 The alcohols do not destroy spores and have a slow germicide activity. Iodine, on the other hand is an oxidising agent that is used as a disinfectant of the skin despite the fact that it is an irritant and is only effective as a sporicide at high concentrations. The ionic and amphoteric agents are odourless antiseptics that do not stain, they are not corrosive to metals and are non-toxic, as well as being stable and cheap. However, they do not act as sporicides or tuberculicides, even at high concentrations. The organo-mercurial compounds, are highly toxic. Hydrogen peroxide is a mild antiseptic, with oxidising and free radical-producing capacity. It is used in gaseous form as a disinfectant of surfaces or for the decontamination of biological cabinets given that it does not possess the toxic and carcinogenic properties of ethylene oxide and formaldehyde. Finally, certain colorants are also used as antiseptics, such as acridine or derivatives of triphenylmethane.

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Among the sterilising agents and/or disinfectants, chlorine and its derivatives, aldehydes, phenolic compounds, and the ethylene oxide can be mentioned.

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Chlorine, hypochlorites and the chloramines are well known disinfectants in the state of the art. The chlorinated product most used in disinfection is sodium hypochlorite that is active against all bacteria, including spores, and is also effective at a wide range of temperatures. The bactericidal activity of sodium hypochlorite is due to the hypochlorous acid (HClO) and to the Cl_2 that is formed when the hypochlorite is diluted in water. Its activity is influenced by the presence of organic material since there may be substances capable of reacting with the chlorated compounds in the medium that diminish their effective concentration and that form organic compounds with carcinogenic properties. Furthermore, chlorine is an irritant and is corrosive to certain materials.

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The aldehydes are alkylating agents that are used as disinfectants and sterilising agents, being sporicides. Glutaraldehyde is the only effective sterilising agent at cold temperatures, but it is fairly toxic and is classified as carcinogenic. Gaseous formaldehyde is used to decontaminate buildings, environments, etc., although it has the inconvenience of being a strong irritant and of losing activity in refrigerated environments.

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The phenolic compounds are commonly used disinfectants. Phenol is not usually used as a disinfectant due to its unpleasant odour, because it is a strong irritant and because of the residue that remains following the treatment of surfaces. The most used phenol derivatives are hexachlorophene and the cresols that are very effective at low concentrations against vegetative forms of bacteria, although they are not effective against spores.

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Similarly, ethylene oxide is a disinfectant agent used in gas sterilisation, generally in the pharmaceutical industry. It serves to sterilise thermolabile material but it is very dangerous due to the fact that it is highly inflammable and explosive,

as well as being carcinogenic.

In the case of infections by prion pathogenic agents, the cleansing, disinfection and sterilisation of the floors and surfaces, or of surgical, hospital or laboratory material, must be performed by chemical decontamination in sodium hypochlorite (2% of free chlorine at room temperature during 1-2 hours) or in sodium hydroxide (1-2 N for 1-2 hours), followed by steam sterilisation in an pre-vacuum autoclave (a cycle at 134 °C during 18 minutes, or six cycles of 3 minutes at 134 °C, for example).

Finally, in order to combat parasites and in particular, insects, mites and arachnids, the current state of the art compounds are of very diverse nature, among which those that should be highlighted: mineral arsenic, fluorinated, sulphur or selenium compounds; compounds based on mineral oils such as anthracene and petrol oils; vegetable compounds such as nicotine, pyrethrin or rotenone derivatives; or synthetic organic compounds such as organophosphorated, organochlorated or carbamate-type compounds. However, many of these have ceased to be used due to their potential toxic effects.

Hence, there is still a need in the state of the art to produce sterilizing, antiseptic, disinfecting and anti-parasitic agents that are not toxic and that as well as acting on a wide spectrum of organisms, particularly micro-organisms including spores, for a very wide range of applications of sterilisation, asepsia, disinfection and deparasitisation of all types of surfaces, objects, areas or organisms.

The dialkylketone peroxides have been known for some time in the state of the art. In particular, the methyl ethyl ketone peroxide is widely known for its industrial use in polymerisation for the curing of unsaturated polyester resins (see for example, the United States patent US 4,931,514, the United States patent application US 2002/0137972 or the international patent application WO 9518180).

Likewise, it is known that methyl ethyl ketone peroxide is used in primer compositions that are applied to substrates (metal, fibreglass, plastic, etc.) that will

be painted (see for example, the European patent application EP 1241233 A).

On the other hand, a composition has been described as a fuel additive that includes a ketone peroxide, such as methyl ethyl ketone peroxide, acetone peroxide or a mixture of both (see the United States patent application US 4,482,352).

Similarly, it is known that compositions including dialkyl ketone peroxide can be used to conserve organic tissues. As such, in the European patent EP 0775439 compositions are described that contain (C1-C6) dialkyl ketone peroxides for the conservation, the anatomical preparation, or the partial regeneration of organic tissues of human or animal origin.

However, the use of dialkyl ketone peroxides *per se* as sterilizing, antiseptic, disinfecting or anti-parasitic agents has not been described or demonstrated.

Surprisingly, the present authors have discovered that dialkyl ketone peroxides can be used *per se* as sterilizing, antiseptic, disinfecting or anti-parasitic agents without any noxious effects, both from the toxicological and environmental point of view, something that is very uncommon among the disinfectants and parasiticides currently known and used in the state of the art, and something that is totally novel in the case of sterilising agents.

The absence of toxicity represents a top-level innovating characteristic, especially in the ambit of sterilisation, in that the few products that are actually commercialised have a very elevated level of toxicity. According to European law, and probably that of all developed countries, the existence of a less toxic product that fulfils the same functions obliges the user to employ it in substitution of the more toxic product. The legislation also foments and promotes the research and development of alternative technologies that reduce the level of danger in the workplace in all areas. It is for this reason that this product has an enormous inventive importance, constituting a clear advance in the existing technology at a worldwide level.

Therefore, the aim of the present invention is to provide the use of said dialkyl ketone peroxides, or of isomers of these compounds, as non-toxic sterilizing, antiseptic, disinfecting or anti-parasitic agents with a very wide spectrum of activity in terms of the type of organisms on which they act (bacteria, virus, fungi, spores, mycobacteria, protozoa, algae, prions, parasites, etc.), and in as much as the type of applications in which they can be employed (human and animal therapies, hygiene, packing, medical and industrial instruments, healthcare environments and sanitary surfaces, premises, general surfaces, industrial installations, refrigeration towers, sanitary hot water circuits, purification of drinking water for human or animal consumption, etc.).

OBJECT OF THE INVENTION

One object of the present invention is to provide the use of a dialkyl ketone peroxide, or an isomers or mix of isomers of this compound, to be used as a sterilizing, antiseptic, disinfecting and anti-parasitic agent.

Another object of the present invention is to provide the use of compositions that include said dialkyl ketone peroxide, or isomers or a mixture of isomers of this compound, to be used as a sterilizing, antiseptic, disinfecting or anti-parasitic agent.

Finally, another object of the present invention is to provide a method of sterilisation, disinfection, asepsia or deparasitisation that involves the application of said composition.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides the use of dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, as a sterilizing, antiseptic, disinfecting and anti-parasitic agent.

In the present invention, the term "sterilizing agent" refers to any chemical substance that eliminates all life forms, including spores. Likewise, the term

“antiseptic agent” refers to any chemical substance that prevents the growth or action of micro-organisms, be it through their destruction or by inhibiting their growth and activity, being a substance that can be applied to the body of a human or animal. Finally, the term “disinfecting agent” refers to any chemical substance that destroys the vegetative forms but not necessarily the resistant forms of pathogenic micro-organisms, being a substance that is applied to inanimate objects.

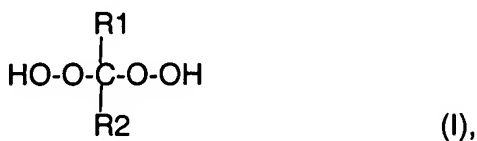
In the context of the invention, the term “anti-parasitic agent” refers to any physical or chemical agent to fight against parasites, eliminating them, repelling them or attracting them, including the products used directly or indirectly for human or veterinary hygiene. In particular, it refers to insecticides, miticides and arachnicides.

All these agents can be considered as biocides, given that they are active substances, or preparations that contain one or more active substances, destined to destroy, or repel, or inactivate the harmful or damaging organisms, to prevent their activity, or to fight against them by any means, through a biological or chemical action.

In one particular embodiment of the present invention, said dialkyl ketone peroxide is employed as a bactericide, virucide, fungicide, sporicide, mycobactericide, protocide, algicide, prionicide, insecticide, arachnicide or miticide.

In accordance with another preferred embodiment of the present invention, the dialkyl ketone peroxide of dealt with can be the (C1-C20) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, preferably of (C1-C6) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same.

In the context of the invention the term “dialkyl ketone peroxide” refers to compounds of the formula (I):

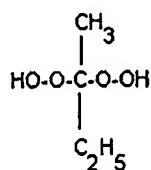


wherein R1 and R2 are the same or different, and they independently represent a (C1-C20) alkyl group, preferably a (C1-C6) alkyl group. Such alkyl groups can be linear or branched, saturated or unsaturated, non-substituted or substituted by diverse organic or inorganic groups.

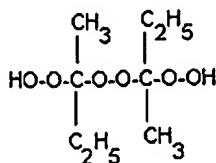
One of the preferred dialkyl ketone peroxides that is encompassed within the present invention is methyl ethyl ketone peroxide, or an isomer or a mixture of isomers of the same.

In the context of the invention, the use of both the dialkyl ketone peroxide itself and an isomer or mixture of isomers of the same is contemplated. The term "isomer" refers to any possible isomer, be it an isomer of polymerisation, a structural isomer or a stereoisomer (an enantiomer, in the case that one or more chiral carbons might exist, or a diastereoisomer), etc.

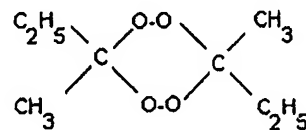
In the case of ethyl methyl ketone peroxide, the following isomeric forms are known:



Monomer



Dimer



Cyclic Dimer

For the dimer, in particular, the D, L and meso- stereoisomers are also known.

In another specific embodiment of the present invention, the dialkyl ketone peroxide described above is employed in very diverse applications in the areas including: human and animal therapy, human and animal hygiene, the washing and disinfection of healthy or damaged skin in man or in animals, packaging, wrappings, medical and industrial instruments, sanitary surfaces and healthcare environments, premises, surfaces in general, industrial installations, refrigeration towers, air

conditioning conduits, machinery and installations in food production, agriculture and fisheries installations, sanitary hot water circuits, purification of drinking water for human or animal consumption, or any other application in the industrial, domestic, environmental, agricultural, forestry, urban, pharmaceutical, civil, military, police enforcement, scientific, technological, spatial, geological, healthcare or health prevention areas for which the usefulness of the biocide properties of dialkyl ketone peroxide are demonstrated.

In human and animal therapy, the dialkyl ketone peroxide can be used as a bactericide, virucide, fungicide, sporicide, mycobactericide, protocide, algicide, prionicide or an anti-parasitic agent, by topical application to infected or infested skin, in distinct pharmaceutical formulations and forms, including those that are mentioned here: pomade, cream, lotion, solution, ointment, powder, solid bar, suspension, emulsion, nebuliser or spray.

Likewise, dialkyl ketone peroxide can also be used as a bactericide, virucide, fungicide, sporicide, mycobactericide, protocide, algicide, prionicide or an anti-parasitic agent, by enteral or parenteral, oral, rectal, vaginal, intramuscular, intradermic, intravenous or intra-arterial application, with the aim of combating infections by bacteria, mycobacteria, fungi, viruses, prions, protozoa, etc., in distinct pharmaceutical formulations and forms, including those that are mentioned here: pomade, cream, lotion, solution, ointment, powder, solid bar, suspension, emulsion, nebuliser, spray, syrup, enema, tablet, capsule, suppository, pessary, elixir or mouthwash.

In human hygiene, the dialkyl ketone peroxide is particularly useful in the formulation of products such as toothpastes and mouthwashes, for example, as an antiseptic at a concentration around 0.25% (v/v), with the additional advantage of its strong bleaching capacity of the dental enamel.

As mentioned above, the dialkyl ketone peroxide can be used as a disinfectant with high level-sterilising capacity to chemically sterilise surgical material that cannot be sterilised thermally, especially endoscopes, as well as the

surfaces of operating theatres and clean rooms. It can also be used to disinfect materials that can be sterilised thermally, the use of such chemical sterilisation offering an alternative method of sterilisation.

5 On the other hand, the dialkyl ketone peroxide can be used as a disinfectant of organic residues, especially of the clinical or sanitary type, before their removal, in order to reduce their levels of infectious toxicity and in this way better comply with the laws concerning the Prevention of Risks in the Workplace and the Laws concerning Hazardous Waste Material.

10 In the same way, another use of the dialkyl ketone peroxide is as an environmental disinfectant in order to disinfect all types of surfaces and non-surgical materials, such as laboratory, food industry, pharmaceutical industry, biotechnologic industry, etc.

15 The dialkyl ketone peroxide has also been employed as an antiseptic disinfectant of healthy or damaged skin (with scars), or as liquid soap disinfectant to wash the hands hygienically with disinfection included. The preparation of this form is carried out by adding the product as an ingredient to a liquid soap.

20 Another of the uses of dialkyl ketone peroxide is as a disinfectant of refrigeration towers against Legionella in refrigeration circuits. Its use consists in the addition of a determined quantity of the product, depending on the volume of water to be treated.

25 A further objective of the present invention is to provide the use of a composition comprising (C1-C20) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, preferably of (C1-C6) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, as has been described previously, in a
30 percentage by volume less than or equal to 50%, and preferably less than or equal to 20%, as a sterilizing, antiseptic, disinfecting and anti-parasitic agent.

In a preferred embodiment of the present invention, the use of a composition

is provided as a bactericide, virucide, fungicide, sporicide, mycobactericide, protocide, algicide, prionicide, insecticide, arachnicide or miticide.

5 In a particular embodiment of the invention, the use of a composition is provided that comprises (C1-C20) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, preferably of (C1-C6) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, as described previously, in a percentage by volume less than or equal to 5%, and preferably less than or equal to 0.3%.

10 In a preferred embodiment of the present invention, a composition is employed that comprises methyl ethyl ketone peroxide, or an isomer or a mixture of isomers of the same.

15 In a preferred embodiment of the present invention, a composition is used that comprises water, any adequate organic solvent, or an oil as an excipient. Among the adequate organic solvents, the alcohols are preferred, and in particular, an alcohol selected from hexylene glycol, polyethylene glycol, propylene glycol, glycerin-formal, diacetone alcohol, ethanol, n-propanol or isopropanol.

20 The preparation of said composition is carried out by conventional methods, by simply dissolving the dialkyl ketone peroxide in the adequate solvent through mechanical agitation, preferably in a reactor for one hour.

25 The methyl ethyl ketone peroxide is commercially available through numerous suppliers at a worldwide level, given that it is a widely used product in industry. One of the commercial products available is Butanox M-50, whose declared concentration of methyl ethyl ketone peroxide is 33% (w/v), always expressed in an approximate form, the remaining 67% being the plasticizer (dimethyl phthalate). Likewise, any other commercial product can be used in which
30 the concentration of peroxide generally, varies between 33 and 50% (w/v), the remaining percentage corresponding to a plasticizer such as dimethyl phthalate or isobutyl phthalate, or 2,2,4-trimethyl-1,3-pentane diol diisobutyrate, for example. The use of this latter plasticizer is particularly advantageous, since it avoids the

possible release of phthalates.

Finally, the current invention provides a method of sterilisation, disinfection, asepsia or deparasitisation that comprises the application of a composition comprising (C1-C20) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, preferably (C1-C6) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, as previously described, with a percentage by volume equal to or less than 50%, preferably equal to or less than 20%, as previously described.

In a particular embodiment, a method of sterilisation, disinfection, asepsia or deparasitisation is provided that comprises the application of a composition comprising (C1-C20) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, preferably (C1-C6) dialkyl ketone peroxide, or an isomer or a mixture of isomers of the same, in a percentage by volume equal to or less than 5%, preferably equal to or less than 0.3%.

In another preferred embodiment, a method of sterilisation, disinfection, asepsia or deparasitisation is provided that comprises the application of a composition comprising methyl ethyl ketone peroxide, or an isomer or a mixture of isomers of the same.

In another particular embodiment, a method of sterilisation, disinfection, asepsia or deparasitisation is provided that comprises the application of a composition that uses water, any adequate organic solvent, or an oil as an excipient. Among the adequate organic solvents, the alcohols are preferred and in particular, an alcohol selected from hexylene glycol, polyethylene glycol, propylene glycol, glycerin-formal, diacetone alcohol, ethanol, n-propanol or isopropanol.

The application of said composition is achieved through conventional methods. In the case of intense disinfection or sterilisation, the mode of employment is the manual immersion in a tank or a similar immersion performed automatically using washing/disinfection machines. In the remaining applications, said composition is employed by bringing the liquid product into contact with the

surface to be disinfected as usual. Among the usual ways to achieve this, the following can be mentioned: nebulization with a nebuliser spray or a spray using propellant gases; dispensation by means of a mechanical device (such as the liquid soaps); pouring with or without dosification onto the hands, skin, an area, piping installation or vessel containing the liquid to be treated; the simple application by extending it with a brush, paintbrush, mop or cloth, or through a dropper, etc.

In the case of the application of the dialkyl ketone peroxide to refrigeration towers in order to prevent or combat the Legionella bacteria, it is recommended to use a 5% dilution of the active ingredient in n-propanol and water, and to dilute it 1:50 such that the active ingredient is applied at the concentration of 0.1% (v/v). Likewise, in order to apply it in sanitary hot water systems, in the system the active ingredient should be diluted to a concentration of 0.1% (v/v). Similarly, for water circuits in the food industry, or for the purification of drinking water, the active ingredient should be diluted to 0.1% (v/v). Finally, for intense disinfection it is recommended that concentrations of 2% be used (v/v).

On the other hand, when referring to the applications for human hygiene (mouthwashes and toothpastes, for example), it is recommended that the dialkyl ketone peroxide be used at a concentration in the order of 0.25% (v/v).

The following examples illustrate the invention.

Example of a topical formulation:

Product 1- NEOSTEX

Antiseptic for the washing of hands

Complete Quantitative Composition

	<u>CAS N.</u>	<u>g/100 ml</u>
Active ingredient:		
Methyl-ethyl-ketone peroxide	1338-23-4	0.25
<u>Other components:</u>		
N-propanol	71-23-8	70
Water	7732-18-5	20
Glycerin	56-81-5	5
Isopropanol	67-63-0	4.5
Menthol	89-78-1	0.25
Dimethyl Phthalate	131-11-3	0.50

Product 2- NEOSTEX PLUS

5 Antiseptic for the washing of hands

Complete Quantitative Composition

	<u>CAS N.</u>	<u>g/100 ml</u>
Active ingredient:		
Methyl-ethyl-ketone peroxide	1338-23-4	0.25
Other components:		
Isopropanol	67-63-0	70
Water	7732-18-5	20
Glycerin	56-81-5	5
N-propanol	71-23-8	4.5
Menthol	89-78-1	0.25
Dimethyl Phthalate	131-11-3	0.50

Examples of activity**EXAMPLE 1****Bactericidal Activity**

Three solutions of methyl ethyl ketone peroxide were prepared at 0.06%, 0.125% and 0.25% (v/v) by diluting Butanox M-50 (approximately 33% methyl ethyl ketone peroxide; w/v) in sterile hard water (300 mg/l of CaCO_3). A neutralising solution of thioglycolate was added at 0.5%, in this way producing three clear and colourless solutions.

Each of the aforementioned solutions was brought into contact with distinct bacterial strains for 5, 15 and 30 minutes at a temperature of 20 °C (*Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 8043 and *Legionella pneumophila* ATCC 33152) and incubated at 20 °C.

The data from the validation assays are shown in Table I and the results of the bactericidal activity of each one of the three methyl ethyl peroxide solutions are shown in Table 1.

TABLE I -Validation Assay

Test organism	Bacterial suspension	Experimental conditions with interfering substance	Toxicity control of the neutralising agent	Control of the method of dilution-neutralisation with the interfering substance
<i>Pseudomonas aeruginosa</i> ATCC 15442	Vc 132; 142 Nv 137	Vc 160; 145 A 152	Vc 121; 124 B 122	Vc 120; 110 C 115
<i>Escherichia coli</i> ATCC 10536	Vc 175; 168 Nv 171	Vc 170; 160 A 165	Vc 160; 157 B 158	Vc 140; 131 C 135
<i>Staphylococcus aureus</i> ATCC 6538	Vc 123; 126 Nv 124	Vc 102; 132 A 117	Vc 115; 107 B 111	Vc 100; 90 C 95
<i>Enterococcus hirae</i> ATCC 8043	Vc 118; 113 Nv 115	Vc 116; 131 A 123	Vc 120; 118 B 119	Vc 115; 100 C 107
<i>Legionella pneumophila</i> ATCC 33152	Vc 135; 160 Nv 147	Vc 127; 140 A 133	Vc 120; 162 B 141	Vc 128; 180 C 154

Vc: viable count;

Nv: number of CFU/ml in the bacterial suspension;

A: number of CFU/ml in the validation assay of the experimental conditions used in this method (with interfering substances);

B: number of CFU/ml in the validation assay of the toxicity of the neutralising agent;

C: number of CFU/ml in the validation assay of the method of dilution-neutralisation.

TABLE 1- Bactericidal Activity

Test organism	Bacterial suspension in the assay	t = 5 min				t = 15 min				t = 30 min			
		0.06% (v/v)	0.125% (v/v)	0.25% (v/v)	0.25% (v/v)	0.06% (v/v)	0.125% (v/v)	0.25% (v/v)	0.25% (v/v)	0.06% (v/v)	0.125% (v/v)	0.25% (v/v)	0.25% (v/v)
Pseudomonas aeruginosa ATCC 15442	Vc 10^{-6} :109;134	2; 2	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0
	10^{-7} : 20; 14	0.2×10^2	0	0	0	0	0	0	0	0	0	0	0
	N 4.8×10^8	24×10^5	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$
Escherichia coli ATCC 10536	Vc 10^{-6} :178; 175	63; 69	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0
	10^{-7} : 16; 26	6.6×10^2	0	0	0	0	0	0	0	0	0	0	0
	N 2.1×10^8	0.3×10^5	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$
Staphylococcus aureus ATCC 6538	Vc 10^{-6} : 160;180	72; 164	35; 18	0; 0	0; 0	115; 120	0; 2	0; 0	0; 0	2; 1	0; 0	0; 0	0; 0
	10^{-7} : 12; 19	11×10^2	2.6×10^2	0	0	11.7×10^2	0.1×10^2	0	0	0.15×10^2	0	0	0
	N 1.5×10^8	0.1×10^5	0.5×10^5	$\geq 10^6$	$\geq 10^6$	0.08×10^5	15×10^5	$\geq 10^6$	$\geq 10^6$	10×10^5	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$
Enterococcus hirae ATCC 8043	Vc 10^{-6} : 240;255	245; 235	176; 180	0; 2	0; 2	240; 256	70; 69	0; 0	0; 0	208; 202	0; 0	0; 0	0; 0
	10^{-7} : 28; 20	24×10^2	17.8×10^2	0.1×10^2	0.1×10^2	24.8×10^2	6.9×10^2	0	0	20.5×10^2	0	0	0
	N 2.4×10^8	0.1×10^5	0.1×10^5	24×10^5	24×10^5	0.09×10^5	0.3×10^5	$\geq 10^6$	$\geq 10^6$	0.1×10^5	$\geq 10^6$	$\geq 10^6$	$\geq 10^6$

Vc: viable count;

N: number of CFU/ml in the bacterial suspension in the assay;

Na: number of CFU/ml in the assay mixture ($< 1.5 \times 10^2$ or $> 3 \times 10^3$ CFU/ml);

R: Reduction of viability (for a bactericidal effect to exist, **R** must be $> 10^5$)

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Conclusion

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The assays performed with the product dealt with in this invention, according to the norm EN 1276 (1997) and at dilutions of 0.06%, 0.125% and 0.25% (v/v) in hard water, demonstrate that said product possesses a bactericidal activity against: *Pseudomonas aeruginosa* within 5, 15 and 30 minutes at the concentration of 0.06%; against *Escherichia coli* within 5 minutes at a concentration of 0.125% and within 15 and 30 minutes at a concentration of 0.06%; against *Staphylococcus aureus* within 5 and 15 minutes at 0.125% and within 30 minutes at 0.06%; against *Enterococcus hirae* within 5 and 15 minutes at 0.25% and within 30 minutes at 0.125%; and against *Legionella pneumophila* within 5 minutes at 0.125% and within 15 and 30 minutes at a concentration of 0.06%.

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EXAMPLE 2

Fungicidal Activity

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Three solutions of methyl ethyl ketone peroxide were prepared at 0.06%, 0.125% and 0.25% (v/v) by diluting Butanox M-50 (approximately 33% methyl ethyl ketone peroxide; w/v) in a sodium chloride/tryptone solution. A solution of thioglycolate (0.5%) was added as a neutralising agent.

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Each of the solutions was brought into contact with the fungal strains *Candida albicans* ATCC 10321 and *Aspergillus niger* ATCC 16404, for 5, 15 and 30 minutes at a temperature of 20 °C and incubated at 30 °C.

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The data from the validation assays are presented in Table II and the results of the fungicidal activity of each of the three solutions of methyl ethyl ketone peroxide are laid out in Table 2.

TABLE II -Validation Assays

Test organism	Fungal suspension	Experimental conditions	Toxicity of the neutralising agent	Validation assay of the method of neutralisation-dilution
Aspergillus niger ATCC 16404	Vc 20; 70	Vc 70; 20	Vc 60; 50	Vc 130; 90
	Nv 4.5 x 10 ²	A 4.5 x 10 ¹	B 5.5 x 10 ¹	C 1.1 x 10 ²
Candida albicans ATCC 10321	Vc 140; 120	Vc 100; 120	Vc 160; 110	Vc 110; 130
	Nv 1.3 x 10 ³	A 1.1 x 10 ²	B 1.3 x 10 ²	C 1.2 x 10 ²

Vc: viable count;

Nv: number of CFU/ml in the fungal suspension;

A: number of CFU/ml in the validation assay of the experimental conditions (with interfering substances);

B: number of CFU/ml in the validation assay of the toxicity of the neutralising agent;

C: number of CFU/ml in the validation assay of the method of dilution-neutralisation.

TABLE 2- Fungicidal Activity

Test organism	Fungal suspension in the assay	t = 5 min				t = 15 min				t = 30 min			
		0.06% (v/v)	0.125% (v/v)	0.25% (v/v)	0.25% (v/v)	0.06% (v/v)	0.125% (v/v)	0.25% (v/v)	0.25% (v/v)	0.06% (v/v)	0.125% (v/v)	0.25% (v/v)	0.25% (v/v)
Aspergillus niger ATCC 16404	Vc	4; 4	2; 1	0; 0	0; 0	2; 3	2; 1	0; 0	0; 0	4; 8	1; 1	0; 0	0; 0
	Na	0.4×10^2	0.1×10^2	0	0	0.2×10^2	0.1×10^2	0	0	0.6×10^2	0.1×10^2	0	0
	R	1.3×10^4	3.6×10^4	$\geq 10^5$	$\geq 10^5$	1.3×10^4	5.5×10^4	$\geq 10^5$	$\geq 10^5$	1.3×10^4	5.5×10^4	$\geq 10^5$	$\geq 10^5$
Candida albicans ATCC 10321	Vc	5; 7	2; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0	0; 0
	Na	0.6×10^2	0.1×10^2	0	0	0	0	0	0	0	0	0	0
	R	2.5×10^4	15×10^4	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$	0.9×10^4	$\geq 10^5$	$\geq 10^5$	$\geq 10^5$

Vc: viable count;

N: number of CFU/ml of the fungal suspension under assay;

Na: number of CFU/ml in the assay mixture;

R: reduction of viability

Conclusion

In accordance with the norm EN 1275 (October 1997), the product that is the subject of this invention possess a fungicidal activity towards the strains mentioned: *Candida albicans* ATCC 10321 and *Aspergillus niger* ATCC 16404.

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EXAMPLE 3

Sporicidal Activity

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Three solutions of methyl ethyl ketone peroxide were prepared at 15%, 20% and 25% (v/v) by diluting Butanox M-50 (approximately 33% methyl ethyl ketone peroxide; w/v) in a sodium chloride/tryptone solution. Each of the solutions was brought into contact with a suspension of *Bacillus subtilis* spores ATCC 19659, for 5, 15 and 30 minutes at a temperature of 20 °C, incubated at 35 °C, and placed on a carrier disc in the presence of mucin and bovine serum albumin.

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Method used: A carrier disc according to the norm ASTM E-2197-02. Standard Quantitative Disk carrier test method for determining the bactericidal, virucidal, fungicidal, mycobactericidal and sporicidal activities of liquid chemical germicides. ASTM International, Pa, USA.

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Suspension of *Bacillus subtilis* spores

ATCC 19659 of the assay8.6 x 10² CFU/ml

Control of the suspension in the presence of mucin8.1 x 10² CFU/ml

Control of the suspension in the presence of bovine serum albumin

7.2 x 10² CFU/ml

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The mean number of colony forming units per ml (CFU/ml) was determined after the exposure of 10 test carrier disks to each concentration of the methyl ethyl ketone peroxide indicated for the time specified. The results of the sporicidal activity of each one of the three solutions of methyl ethyl ketone peroxide are shown in Table 3.

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TABLE 3 – Sporicidal Activity

Concentration of peroxide (%)	Exposure time		
	5 min	15 min	30 min
15%	7.2×10^2	4.3×10^2	1.8×10^2
20%	7.6×10^2	0.5×10^2	0
25%	5.0×10^2	0.3×10^2	0

Conclusion

The product that is dealt with in this invention, when used at the concentration of 20% and 25% during 30 minutes, possesses absolute sporicidal activity under the specific conditions of this assay. When used during 15 at a concentration of 20% and 25%, the sporicidal activity is not absolute although it significantly reduces the number of viable spores.

EXAMPLE 4**Virucidal Activity**

A solution of methyl ethyl ketone peroxide was prepared at 0.25% (v/v) by diluting Butanox M-50 (approximately 33% methyl ethyl ketone peroxide; w/v) in cell culture medium. This solution was brought into contact with a suspension of poliovirus type 1 ATCC VR-192 for 15 minutes at a temperature of 20 °C; (incubation temperature: 35 °C).

The Method utilised: ASTM E-1053-97, is a Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces. ASTM International, Pa, USA.

- Suspension of Poliovirus type 1 ATCC VR-192 assayed..... 1×10^7 TCID₅₀
- Cell line usedVero cells.
- Control of the poliovirus type 1 suspension used under the assay conditions without exposure to disinfectant, with titre in base 10 in order to calculate the

TCID₅₀ units. Four replicates per dilution.

- Control of cytotoxicity of the disinfectant: observations of the effect on each cell monolayer inoculated with disinfectant at dilutions in base 10 and intermediates in base 2. Four replicates per dilution.
- Control of the cell monolayer, four replicates over 4 days of observation.
- Method of disinfectant neutralisation: dilution in cell culture medium until it is no longer cytotoxic: 1:7,000 with four replicates.

The mean number of infective units per ml (TCID₅₀) for a cell culture was determined after exposing the 10 monolayers to each of the concentrations of the product of the invention for the time indicated. The results of the virucidal activity of the solution of methyl ethyl ketone peroxide are shown in Table 4.

TABLE 4- Virucidal Activity

PRODUCT CONCENTRATION (%)	Exposure time		
		15 min	
0.25% in cell culture medium		Absence of CPE Reduction (log ₁₀): ≥ 5	
CONTROLS			
Control viral suspension without disinfectant		1x10 ⁴ TCID ₅₀	
Control of cytotoxicity		Absence of CTX	
Control of cells		Normal cells	
Control of the neutralisation of the disinfectant		Normal cells	

CPE: cytopathic effect;

CTX: cytotoxicity.

Conclusion

The product dealt with in this invention possesses an absolute virucidal effect at a concentration of 0.25% within 15 minutes, with a reduction > 1 x 10⁴ TCID₅₀ against Poliovirus type 1 under the conditions indicated.

EXAMPLE 5**Mycobactericidal Activity**

Three solutions of methyl ethyl ketone peroxide were prepared at 1%, 2% and 4% (v/v) by diluting Butanox M-50 (approximately 33% methyl ethyl ketone peroxide; w/v) in a solution of sodium chloride-tryptone. Each of these solutions was brought into contact with a suspension of *Mycobacterium terrae* ATCC 15755 for 5, 15 and 30 minutes at a temperature of 20 °C, incubated at 35 °C and then placed on a carrier disk in the presence of mucin and bovine serum albumin.

The method used: ASTM E-2197-02. Standard Quantitative Disk carrier test method for determining the bactericidal, virucidal, fungicidal, mycobactericidal and sporicidal activities of liquid chemical germicides. ASTM International, Pa, USA.

-Suspension of *Mycobacterium terrae* ATCC 15755 in the assay2,100 CFU/ml
 - Control of the suspension in the presence of mucin1,700 CFU/ml
 - Control of the suspension in the presence of bovine serum albumin 1,850 CFU/ml

The mean number of colony forming units per ml (CFU/ml) recovered was determined after the exposure of the 10 test carrier disks to each one of the three test solutions of methyl ethyl ketone peroxide indicated during the time stipulated.

TABLE 5- Mycobactericidal Effect

Concentration of peroxide (%)	Exposure time (minutes)		
	5 min	15 min	30 min
0.5%	175	100	90
1%	160	0	0
2%	0	0	0
4%	0	0	0

Conclusion

The product dealt with in this invention possesses a mycobactericidal effect

at a concentration of 1% within 15 and 30 minutes, and at the concentrations of 2% and 4%, the mycobactericidal effect can be seen at 5, 15 and 30 minutes.